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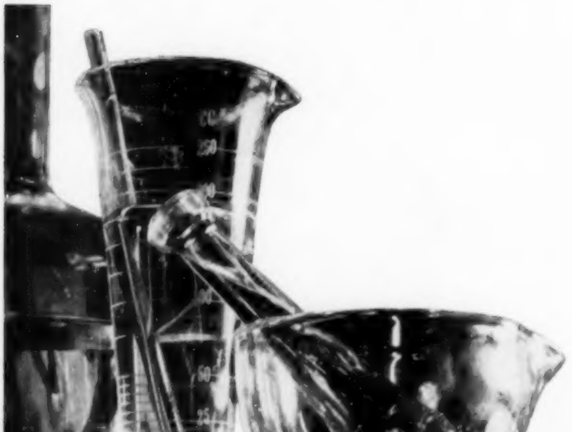

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# AMERICAN JOURNAL OF PHARMACY

AND THE SCIENCES SUPPORTING PUBLIC HEALTH

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# E D I T O R I A L

## A PROFESSIONAL CRITERION

ONE of the greatest weaknesses in the organization of pharmacy as a profession is its failure to properly police itself. Someone has quite aptly stated that the true measure of a profession lies in its willingness and ability to regulate itself from within. To this, we firmly subscribe and it would seem that our profession might well take some sorely needed steps in this direction.

Quite unlike most of the other true professions, pharmacists look to various state and federal laws as a means of eliminating or curtailing those practices which are deemed improper. In fact, pharmacists have at times become proponents of legislation to accomplish this end. This places us in the rather unique and somewhat untenable position of publicly confessing that we cannot regulate ourselves and require the surveillance of some outside group, usually with police authority, to see to it that we follow those dictates of professional behavior which all of us know full well would be proper.

The medical profession, in contrast, attempts to regulate itself through the proper functioning of grievance committees before whom evidence of alleged professional misconduct can be presented by both physicians and the laity. In the event a physician is found guilty of unethical practice, he can be barred from his county medical society and, with this, membership in the state and national groups as well. Such action deprives him of the right to practice in many hospitals and so injures his professional status that his functioning as a physician is placed in serious jeopardy. Priests and ministers in a similar manner can be called before a tribunal of their church to explain any alleged misconduct making them unworthy of their calling, and it does not take evidence of breaking some state or national law to bring this about.

In pharmacy, we are blessed or cursed—depending on one's viewpoint—with more state and national laws governing our practices than any other profession. A pharmacist almost never is expelled from a pharmaceutical organization for a transgression of these laws or the code of ethics propounded by the organization. What is even

worse, boards of pharmacy who presumably have the authority to revoke the license to practice do so only under the most flagrant and repeated evidence of illegal acts. Board members seem quite loath to take a fellow pharmacist to task for reasons which stem neither from charitable instincts nor love of their profession. The reason that boards rarely take definitive action is because they are not urged to do so by the rank and file of pharmacists, together with the fact that board appointments are usually political and pressure is often applied to assist the pharmacist who is guilty of malpractice in evading punitive action.

The profession of pharmacy is, and will continue to be, exactly what pharmacists by and large want it to be. It does little good for pharmacists to raise funds to improve public relations to convince the laity of their professional status and to do many other such things unless there exists in the minds and hearts of pharmacists generally the desire for greater professional integrity and the insistence that other pharmacists not treat this integrity lightly. We can and should police ourselves. It is not an impossible task. To exactly that degree that we work toward this end, we will merit and receive public acceptance of our professional status.

L. F. Tice

# SELECTED NEWER DEVELOPMENTS IN THE EVALUATION OF GERMICIDES *

## (Part II)

### (Concluded from May issue)


By Emil G. Klarmann **


### Sporicidal Action of Disinfectants

THE problem of sporicidal action of chemical agents has been the subject of bacteriological studies for many years. To propound the question as to whether or not it is possible to effect sporicidal action by such means, is to ask whether "chemical sterilization" is feasible, the term "sterilization" being used in this case in its all-inclusive meaning, i.e., denoting the elimination from the sterilized object of all microbial life and especially of resistant bacterial spores. This is in contrast to "disinfection", a term which does not encompass sporicidal action.

It would not be feasible, within the framework of this presentation, to review the great number of papers published on the subject of "chemical sterilization". Suffice it to say, that various chemical agents have been credited, over a period of time, with the capacity of killing bacterial spores, beginning with Koch who thought that anthrax spores could be killed by a 1/10 per cent solution of mercuric chloride. Subsequently, other chemical agents were claimed to be possessed of a sporicidal potential.

Incidentally, Reddish and four collaborating laboratories have shown that heating bacterial spores to 80°C. for 10 minutes (intended to eliminate the vegetable cells) injures them to the extent that they are more easily killed by chemicals than unheated spores. It follows that sporicides should always be tested with unheated mature, pathogenic spores, especially those of high resistance, such as *B. anthracis, C. tetani,* etc.

As of the present, it would seem that direct claims for a sporicidal effect at room temperature, are being made most prominently

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* Presented at the Symposium on Analytical Microbiology, 56th General Meeting of the Society of American Bacteriologists, Houston, Texas, May 3, 1956.



** Lehn & Fink, Inc., New York, N. Y.

on behalf of certain formaldehyde preparations, although one also meets occasionaly with pertinent representations made for some quaternary ammonium compounds, and for iodophores. (Of course, no consideration is given here to chemicals which might be able to sterilize spore-bearing objects but would be corrosive to metals and, therefore, useless, e.g., as instrument germicides.)

That quaternary ammonium compounds are not capable of killing the spores of resistant Clostridia and of the anthrax bacillus has been stressed recently by Klarmann and Wright (25) who also suggested that published allegations to the contrary were based upon faulty testing techniques, and particularly upon failure to control sporostasis in the subcultures. As to the action of formaldehyde on bacterial spores, two recent papers originating in the laboratory of a regulatory government agency are of considerable methodological interest. The paper by Ortenzio, Stuart and Friedl (26) describes a procedure for obtaining spores of the desired resistance, and for exposing them to the action of the disinfectants to be evaluated; the subsequent paper by Friedl (27) reports upon a study of the reproducibility of the sporicidal test. As may have been expected from available published information, a cresylic disinfectant (tested in the dilution of 1:64) was unable to kill the spores of *B. subtilis* or *C. sporogenes* in two hours. A formaldehyde type germicide (tested in undiluted form) appears to have been sporicidal for *B. subtilis spores* in 30, but not in 10 minutes; spores of *C. sporogenes*, however, were not killed in 2 hours which was the longest time of exposure employed.

This finding as to the absence of sporicidal action upon *C. sporogenes* spores suggests that the formaldehyde germicide tested may be possessed of inhibitory properties which should be controlled by means of sub-transfers. Recently, we carried this work forward in our laboratory in an endeavor to answer the question as to whether or not exposure for more than 2 hours would kill resistant bacterial spores of the type under discussion (28). We found that by introducing the simple expedient of the addition of serum to the subtransfers, we obtained survival of spores of *C. sporogenes, perfringens* and *tetani*, even after 8 hours, in several instances. Concerning the role of serum in this picture, we are not prepared to commit ourselves at this moment as to whether this material acts by virtue of enriching the culture medium (rendering it recuperative for the damaged

spores), or by neutralizing any inhibitory traces of the germicide transferred into the subcultures.

Substantially, the same findings were obtained with an 8 per cent aqueous formaldehyde solution, and with a formaldehyde germicide containing hexachlorophene. As to the latter, there is no reason to believe that hexachlorophene contributes significantly to the sporicidal performance of the preparation since it is essentially bacteriostatic in action, and since moreover, this action is subject to reversal by contact with tissue fluids (30). (However, it is possible that hexachlorophene contributes to the *sporostatic* effect of the combination.) Similar observations were made when using steel rings ("penicillin cups") as carriers for *C. sporogenes,* and with blood serving as the inactivating agent. In these tests, too, a significant number of the carriers did not appear to be sterilized after an 8 hour exposure to an 8 per cent formaldehyde solution.

At any rate, the use of serum or blood in a test of this kind is held to be entirely rational and proper since formaldehyde germicides are used extensively for the chemical "sterilization" of surgical instruments which subsequently come in contact with tissue fluids showing a comparable or probably superior "neutralizing" capacity for the inhibitory traces of the germicide adhering to any spores that may be present upon such instruments.

It was found also that *B. subtilis* spores while being less resistant to the action of formaldehyde than the spores of the three Clostridia, nevertheless survived a 4 hour exposure, provided that the factor of sporostasis was controlled. This finding, too, is in contrast to the collaborative report of Friedl according to which *B. subtilis* spores are killed in not more than 30 minutes (and in less than that, in some cases); again, it suggests that the sub-transfer technique referred to in Friedl's report may not be adequate to eliminate sporostasis, in this instance.

All of this is not intended to detract from the value of the work of Friedl, Stuart and associates which aims at achieving a standard method for the evaluation of disinfectants claimed to be capable of sporicidal action; on the contrary, it appears that with due consideration given to the rational control of sporostasis, a satisfactory technique may yet be developed.[2]

2. Recently, this method has acquired a quasi-official status by virtue of its publication in the eighth edition of the "Official Methods of Analysis of the A. O. A. C."

While carrying out this work in our laboratory, we demonstrated incidentally that sporicidal action is produced at boiling temperature by dilute solutions of certain non-volatile synthetic phenolic disinfectants within a practical period of time, viz., of the order of 10 to 15 minutes. As is known, some spores will resist the action of plain boiling water for several hours (25).

An iodophore preparation tested (with 1.6 per cent of available iodine), did not kill *C. sporogenes* spores in 8 hours, in any practical dilution; this agrees with the observation that whereas undiluted Strong Iodine Solution U. S. P. is sporicidal in 2 hours (or possibly less), dilution with an equal volume of water impairs the sporicidal action very markedly in that the effect is obtained only in 8, but not in 4 hours.[3]

## Note on "Neutralizers"

Since an issue has been made in several instances of the need for the proper use of the right kind of "neutralizers" in disinfectant testing, in order to prevent the risk of mistaking bacteriostasis for bactericidal action, some additional amplifying comments are deemed to be relevant to this matter, especially in view of the numerous publications containing a variety of suggestions as to suitable materials. If one is concerned primarily with the practical aspects of this problem, than obviously only those materials should be used as "neutralizers" which are likely to occur at some significant point of a given disinfectant's intended use so as to justify an inquiry into the possibility of its interfering with the action of this disinfectant; the same consideration will not apply in the case of a purely investigational study where some particular antagonists, which are not normally

3. *Addendum re Virus Hepatitis.* The proper sterilization of appliances, particularly of syringes and needles is highly relevant to the prevention of infection with hepatitis viruses A and B carried by human blood or some of its products (such as serum, plasma, fibrinogen, etc.), when administered by transfusion or injection. Since, at present, only man is known to be susceptible to infection by either virus, there are no laboratory methods available which would permit the evaluation of sterilizing effectiveness of any chemical disinfectant with respect to these viruses. The "Expert Committee on Hepatitis" of the World Health Organization (Tech. Rept. No. 62, Geneva 1953) considers the following procedures to be adequate: boiling in water for at least 10 minutes, autoclaving, or treatment in hot-air oven (for one-half hour at 170°C., if the temperature can be controlled in all parts of the oven, otherwise treatment for one hour at 180°C.). No chemical disinfectants (acting at room temperature) are considered acceptable by this committee for the purpose in question.

found under actual use conditions, may be employed to answer a question not related to a product's practical performance. In this connection, it should be remembered also that whereas some disinfectant chemicals act as gross protoplasmic poisons, others may produce the desired effect by merely inactivating some essential biological function or functions of the bacterial cell. While there is no question of the infectious agent being put *hors de combat* in the former case, there is the question, in the latter case, as to whether or not the microorganism which *presumably* has been killed or otherwise rendered harmless, has *really* ceased to be a source of infection when placed under the more favorable conditions to be found, e.g., in an open wound, on a mucous membrane, in the intestinal tract, etc., as the case may be with respect to the particular pathogen and the particular surroundings favorable for its proliferation (30).

Incidentally, insofar as the testing procedure itself is concerned, a distinction should be drawn also between that portion of the antibacterial agent which may have been "transferred" with the inoculum to the subculture medium in a quantity sufficient to produce bacteriostasis, and that portion which upon becoming affixed to the bacterial cell in the course of the disinfectant process would have initiated a series of reactions ultimately leading to the abolition of its infectiousness, unless interrupted by some antagonistic matter (furnished by the animal body) acting upon the "injured" cell so as to reverse the effect of the antibacterial substance. Of course, both processes viz., neutralization and reversal may take place at the same time; except for a special experimental set-up, the testing procedure will not usually answer the question as to the control of either one or of both of these factors.

## Antiseptics

While for the evaluation of disinfectants in their different areas of usage several methods are available which enjoy an "official" status through endorsement of a governmental regulatory agency, no such situation exists in the case of antiseptics. The one thing which is official here is the meaning of the term "antiseptic", since this term has been defined by no less an authority than the Federal Food, Drug, and Cosmetic Act of 1938; according to this definition, an "antiseptic" is considered to mean the same as a "germicide", except where a prolonged contact with the body is assured (as, e.g., with an ointment,

a wet dressing, etc.) in which case the "antiseptic" may display an inhibitory rather than a germicidal action.

As with disinfectants, so also here there is an area in which the terms "inhibitory" and "germicidal" are relative in that a permanent inhibition of the reproduction of bacteria will lead ultimately to their death. By the same token, the observation made in the case of disinfectants, as to the restoration of bacterial viability through neutralization or inactivation of the bacteriostatic principle, e.g., by a favorable contact of the affected cell with certain body fluids (blood, serum, etc.) will apply with the same force here.

At one time, the term "antiseptic" had a broader meaning; nowadays it is used almost exclusively for preparations intended for application to living tissue.

In 1931 there appeared a publication, authored by Ruehle and Brewer and entitled "United States Food and Drug Administration Methods of Testing Antiseptics and Disinfectants". The several methods prescribed therein for the examination of antiseptics were developed mostly by Reddish. They enjoyed, for some time, a quasi-official status, having originated with a governmental authority. They are still useful for a variety of purposes, such as a first screening of new preparations designed for use as antiseptics, i.e., prior to their more extensive clinical evaluation under the practical conditions of their intended application. However, the Food and Drug Administration (now a part of the U. S. Department of Health, Education and Welfare) no longer encourages the designation "Food and Drug Administration" or "F. D. A." methods; according to its present position, there exist no standard or official tests for the evaluation of antiseptics, and no single *in vitro* test is considered sufficiently informative to serve as a criterion of presumed capacity of performance under the conditions of practical use.

It appears actually impossible to devise any single representative method which would cover the whole range of circumstances under which antiseptics are used. Thus, obviously, the same criteria of performance should not be applied to an antiseptic mouth-wash as are applied to a first-aid antiseptic (i.e., for use on wounds, abrasions, etc.). More comprehensive information is elicited by a "profile evaluation" involving the combination of several different tests, with every test supplying an answer to a particular pertinent question.

To the extent that *M. pyogenes* var. *aureus* represents the most common cause of suppuration, coupled with marked resistance to both

physical and chemical factors, its use as test-organism is logical in screening tests by means of one of the several applicable Reddish methods. However, with specific reference to antiseptics applied for the purpose of preventing infection through a break in the skin, an *in vivo* method such as, e.g., that of Nungester and Kempf (29) may yield more relevant results.

Another recent development, in the study of oral antiseptics, is the use of selective media for determining changes in the oral flora following the use of such preparations. For this purpose, the following media have been proposed by Watson and Reddish: (1) tomato juice agar for aciduric microorganism, (2) Rogosa agar for lactobacilli, (3) mitis salivarius agar for streptococci, (4) Chapman-Stone agar for staphylococci, (5) malt extract agar for yeasts, and (6) NIH agar for general counts. These media have proved quite valuable in following the changes in the microbial flora as a result of the use of antiseptics in the oral cavity.[4]

## "Semi-Micro" Method and Results

Klarmann, Wright and Shternov (30) reported that the "semi-micro" method referred to previously, is capable of furnishing more informative data as to the performance of liquid antiseptics likely to come in contact with tissue fluids. One of the pragmatic features of this technique is control of the random sampling error inherent in methods which depend upon loop transfers from the "medication mixture" to the "sub-culture". In addition, its use considers the following premises:

1. The criterion of fitness of any antiseptic which is likely to come in contact with tissue fluids should be its demonstrated capacity for permanent suppression of bacterial activity under the conditions of use;

2. If an antiseptic which has prevented bacterial proliferation in a nutrient medium does not continue to prevent it upon subsequent contact with physiological material such as blood or serum, then its fitness for use as a pre-operative or wound antiseptic is open to question.

The procedure consists substantially of the following steps: A broth culture of *M. pyogenes* var. *aureus* is exposed to the action of

4. Paper presented at the meeting of the American Pharmaceutical Association in Detroit, 1956.

the antiseptic for ten minutes, at room temperature. The reaction is stopped by dilution with about forty times the original volume of blood broth. The total mixture is incubated, and the results are read after 96 hours.

The results in the following Table V reveal marked differences in the behavior of the several types of antiseptic agents tested.

TABLE V

MINIMUM GERMICIDAL CONCENTRATIONS BY SEMI-MICRO METHOD
Test Organism: *Micrococcus pyogenes* var. *aureus*, 20°C.

| Antiseptic | A.O.A.C. Broth | A.O.A.C. Broth + 10% Blood |
|---|---|---|
| Phenol | 1:50 | 1:50 |
| Cresylic Disinfectant (50% cresylic acid) | 1:150 | 1:125 |
| Synthetic Phenolic Disinfectant (p.c. 5) | 1:100 | 1:100 |
| Hexachlorophene | 1:40,000* | 1:400* |
| Benzalkonium chloride | 1:22,500* | 1:2000* |
| Benzethonium chloride | 1:25,000* | 1:5000* |
| Hypochlorite Solution (0.9% av. Cl) | 1:70 | 1:50 |
| Strong Iodine Solution, U. S. P. | 1:300 | 1:300 |
| Brilliant Green | 1:50,000* | 1:2000* |
| Azochloramid | 1:550 | 1:550 |

* Bacteriostasis.

Test-organisms exposed to the action of the minimum germicidal concentrations of phenol and of the phenolic disinfectants appear to have been rendered non-viable, also with respect to their subsequent contact with 10 per cent blood broth.[5]

Non-viability of the exposed test-organisms characterizes also the performance of the hypochlorite solution, of azochloramid and of the stronger Iodine U. S. P.

On the other hand, hexachlorophene appears to be markedly affected in its action, and the same is true of the three quaternary ammonium compounds, benzalkonium chloride, benzethonium chloride and cetyl pyridinium chloride. These factors should be borne in mind in connection with any prophylactic application to broken skin in-

5. The synthetic phenolic disinfectant (p. c. 5) contains as active phenolic component o-phenylphenol. It contains potassium ricinoleate as emulsifying agent.

tended to protect against infection, either unintentional (as, e.g., in the case of an injury) or intentional (as in pre-operative use). They are probably of no significance in connection with some uses in which suppression of the bacterial flora is desired on the unbroken skin (as, e.g., in the case of a deodorant preparation).

Brilliant Green which exhibits marked bacteriostatic capacity also suffers a strong impairment in its performance, under the conditions of this test.

Incidentally, it should be noted that the several agents which exhibit such marked bacteriostasis upon dilution with plain A. O. A. C. broth continue to show bacteriostatic action upon dilution with 10 percent blood broth; possibly higher blood concentrations would be required in some or all of these cases to suppress bacteriostasis completely.

In tests with three organic mercurials (Merbromin 1:50, Nitromersol 1:2500, Thimerosal 1:1000, all three in aqueous solutions), and with mercuric chloride, it was noted that the negative results observed in plain broth were due to the inhibition of growth of *M. pyogenes* var. *aureus* rather than to any germicidal action; however, with the aid of 10 per cent blood broth, bacteriostasis was suspended and growth was obtained. It is of interest that in this respect blood and thioglycolate produced comparable results while serum appeared to be ineffective; of course, thioglycolate has been specified for some time as a neutralizing agent in tests on mercurial antibacterial agents.

The reversal of inhibition by mercurials in the presence of blood may be said to be of eminent practical significance with respect to the use of these chemicals in first aid, as well as in pre-operative prophylaxis.

It should be added parenthetically that, with reference to the *modus operandi* of the "semi-micro" method as applied to the screening of antiseptics, the assay is not conducted in the presence of blood; rather, the test-organism is given the opportunity of coming in contact with blood following exposure to the antiseptic under study. While this procedure may be illustrative of the existence of any reversing action upon growth inhibition by the antiseptic, it is also less severe with respect to its comparability with actual conditions of use; here an antiseptic may have to act in the presence of blood (or other tissue fluids) from the moment of its application, thus possibly suffering an impairment of its action by the organic matter encountered,

as well as by any reversal of the effect of that portion which has entered into some form of inhibitory combination with the bacterial cell.[6]

## Note on "Degerming" Agents

While the so-called "degerming" agents are not, strictly speaking, in the realm of antiseptics, brief comment may be in order concerning their testing methodology, particularly with reference to what amounts to their antiseptic usage.

The highly favorable reports published originally on the subject of hexachlorophene bearing soaps and other detergents, have been followed recently by more critical ones. While there is no disagreement as to the validity of the method of testing such preparations as developed originally by Price (31), or modified by some later investigators (32), the interpretations of the findings obtained by such methods is open to discussion, particularly in the area of their surgical and other medical applications.

There is no doubt that regular use of "degerming" agents will bring about a reduction of the "resident" bacterial flora of the cutaneous surface although there are other, possibly more effective means of achieving this effect, particularly in its surgical aspects. In any case, it does not appear feasible by this means to control subsequent contamination with "transient" pathogens, or to eliminate the risk of skin lesions of bacterial origin. For details on this and related subjects, reference should be made to the papers by Blank (33, 34, 35) and Price (36).

It should be added, for the sake of completeness, that "degerming" agents of the type of hexachlorophene and bithionol, while inhibitory for gram-positive microorganisms, are much less effective against gram-negative ones. Of course, such information should be available to surgeons and operating room personnel.

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6. Thus, with respect to several inorganic and organic mercurials, Miller and Rose (48) showed that *in the presence* of blood (at certain critical levels which are different for each compound tested) antiseptic action is no longer in evidence *in vitro*. Brewer's (49) observation as to the virtual elimination of infectivity of certain pathogens, following their exposure to merbromin, does not necessarily furnish adequate evidence in support of an argument for the practical, antibacterial performance of mercurials, particularly in wounds where the presence of blood may be a factor.

## Comment on "Tissue-Toxicity" Tests

It is not deemed necessary, for the purpose of this presentation, to dwell upon the determination of the "tissue-toxicity" of antiseptics at any great length. Although various pertinent testing methods have been suggested by Salle, McOmie and Schechmeister (37), Nye (38), Welch, Slocum and Hunter (39), Russell and Falconer (40), and others, it is held that the problem itself is not necessarily of primary practical importance with respect to the task whose accomplishment is expected of an antiseptic worthy of this designation. Incidentally, some of the results obtained by one or another of these methods do not permit of a rational practical utilization which would serve as the basis of a comparative evaluation of antiseptics.

This is not to say that, other things being equal, an antiseptic with lower "tissue-toxicity" should not be given preference over a more toxic one; however, such an axiomatic statement is predicated upon the availability of a testing procedure which would be more representative of the conditions of actual usage than the test methods published to date. As matters stand now, one may even properly concede the possibility of an antiseptic injuring superficial tissue, or inactivating some leucocytes present locally, so long as such a negative action is merely of a temporary character while at the same time involving the promise of effective control of the infectious agent itself, i.e., the prevention of its penetration to a depth where it could not be reached following prompt application of the antiseptic to the contaminated wound.

Parenthetically speaking, the problem of "tissue-toxicity" appears to be of considerably greater importance in the area of chemotherapy than in that of antiseptics.

## Antifungal Agents

Only brief reference can be made here to testing methodology as applied to antifungals.

Inasmuch as control of pathogenic fungi may be desired either on inanimate surfaces or on the human (or animal) body, different procedures will apply to the evaluation of preparations intended for the one or the other purpose.

It would not be feasible to give here a review of the several methods for assaying antifungal performance on inanimate objects which have been advanced over a period of years. Proceeding again

in an eclectic manner, mention should be made of the method of Burlingame and Reddish (41) which employs five pathogenic fungi most commonly associated with "athlete's foot". (Any one of these fungi, or a combination of two or more may be involved in actual cases of dermatophytosis). This method does not permit a direct differentiation between fungicidal and fungistatic action; accordingly, the presumptive evidence obtained by it is for fungistasis only.

Klarmann, Shternov and Costigan (42) developed an adaptation of the phenol coefficient technique to the evaluation of fungicides. A safety factor of significant magnitude is provided by the requirement for using a number of fungi which is some 5000 times greater than the number found on the floors of shower and locker rooms, or in foot pans. Either *Trichophyton rosaceum* or *Trichophyton interdigitale* (*gypseum*) are specified as test-organisms; as to the identity of the former, Emmons (43) expressed the view that a saprophytic fusarium rather than a pathogenic fungus may be involved here.

The compendium "Official Methods of Analysis of the A. O. A. C." (eighth edition 1955) lists a "fungicidal test" which employs the principle of the phenol coefficient technique. The test is backed by collaborative data collected and interpreted by Ortenzio (44).

It was mentioned before that the "Square Diluent" testing method of Stedman, Kravitz and Bell (19) calls for the use of a pathogenic fungus together with two bacterial pathogens, as test organisms.

The principle of the "semi-micro" technique lends itself for adaptation to the evalution of antifungal agents, as shown by Klarmann and Wright (45). When studied by this method (with "Bacto-Oxgall" serving as the "neutralizer"), the seven quaternary ammonium compounds tested were found to be practically devoid of fungicidal action in any practical dilution with respect to *Trichophyton interdigitale* and *Trichophyton rubrum*.

However, it might be pointed out here that the problem of infection by pathogenic fungi resulting from contact with contaminated inanimate objects (floors, mats, etc.) does not seem to be quite as urgent as has been thought until recently. Experimental and clinical evidence gathered by Baer (46) and associates suggests rather forcibly that in healthy individuals the skin does not normally become infected with fungi in this manner; what is experienced by the individual as a fungus infection is actually an exacerbation of a dormant or latent

condition brought about by climatic, physiologic, immunologic (allergenic), therapeutic or other factors favoring such a development.

The comments made up to this point apply essentially to antifungal agents for use as "fungicidal disinfectants", i.e. on inanimate objects. As to products available for use on the living body, i.e., for therapeutic purposes, their number and diversity is such as to raise serious doubt regarding the general relevance of any one of the numerous screening procedures published. Another complicating factor is introduced by the discrepancy between the demonstrated *in vitro* antifungal efficacy, and the clinical or practical desirability of any given preparation. For a consideration of these and other pertinent matters reference should be made to the chapters on "Fungistatic and Fungicidal Test Methods" and "Fungistatic and Fungicidal Compounds" by Oster and Golden (in "Antiseptics, Disinfectants, Fungicides and Sterilization", edited by G. F. Reddish, 1954.)

## The Problem of Virucidal Disinfectants

A non-specific disinfectant is not necessarily a non-specific virucide, some authoritative opinion to the contrary notwithstanding (50). There appears to be substantial agreement to the effect that a general purpose disinfectant should be a non-specific germicide, effective under practical conditions of use, against the entire spectrum of vegetative pathogens, viz., enteric, respiratory and dermal; preparations exist which fulfill or at least approach this requirement. By contrast, there is as yet no agreement as to the delineation of the activity of a virucide; while the number of pathogenic viruses is probably greater than that of pathogenic bacteria, no agreement has been reached as to which viruses must be inactivated by a given substance before it merits the designation of a virucide.

At this time, it is apparent that some of the most valuable germicides may not have the capacity of eliminating the infectiousness of some of the most important viruses (such as that of poliomyelitis), under practical conditions of use; moreover, the differences in susceptibility to inactivation by various antiviral agents of the several viruses appear to be as great as, or even greater than the corresponding differences observed in the case of bacteria.

While this is not the place to deal with the biological characteristics of viruses or their mode of proliferation, it should be remembered that, unlike bacteria, viruses are metabolically inert entities

which depend upon specific types of the invaded living host cells to supply the wherewithal for their multiplication; this they accomplish by altering the parasiticized cell's metabolism so as to force the production of new viral material (51).

But here the analogy between the several viruses comes to an end. The great chemical differences between them are illustrated by the finding that e.g. in the case of influenza virus, protein material accounts for less than 50 per cent of the dry weight while in the case of rabbit papilloma virus the figure is over 90 per cent; also, while some 50 per cent of the former consists of lipids, less than 6 per cent of vaccinia virus (dry weight) is of this character (52). Such great differences in composition must be expected to find expression in the wide qualitative and quantitative variations in susceptibility to inactivation by chemical and physical agents.

As in the case of bacterial pathogens, so also in the case of viruses such inactivation has its theoretical and its practical aspects. From the former point of view, it is of great interest, for a variety of reasons, to isolate the different viruses in their pure form, i.e., substantially free from any accompanying organic matter; among other things, this operation would permit the study of the direct action of various inactivators, also the determination of the concentration ratio of virus to inactivator in any individual case. However, from the viewpoint of practical disinfection, the situation here corresponds to that encountered in the case of bacteria, in that both viral and bacterial pathogens create sanitation problems while occurring in the presence of organic matter which, as a rule, will play a minor or a major part in protecting such pathogens against the effects of the sanitary procedures directed toward their elimination as infectious agents.

By way of illustration, the following Table VI compares the action of several disinfectants upon the influenza A and Newcastle viruses, as observed in our laboratory.

It is found that in the case of the two synthetic phenolic disinfectants, the germicidal concentrations (as derived from the A. O. A. C. phenol coefficients and confirmed by the "Use-Dilution" test) are also virucidal for the two test viruses; by contrast, the iodophore compound (with 1.6 per cent of available iodine) was active only in a 10 per cent solution (corresponding to 1600 parts per million of available iodine) against influenza A virus, and in a 4 per cent solution (corresponding to 640 ppm av. I) against Newcastle virus.

TABLE VI

INACTIVATION OF INFLUENZA A AND NEWCASTLE VIRUS *

| *Disinfectant (Active Ingredients)* | *Dilution* | *Influenza A Virus* Eggs Injected | *Influenza A Virus* Hemagglutination + | *Influenza A Virus* Hemagglutination — | *Newcastle Virus* Eggs Injected | *Newcastle Virus* Hemagglutination + | *Newcastle Virus* Hemagglutination — |
|---|---|---|---|---|---|---|---|
| o-Phenylphenol (p.c. 5) | 1:100 | 6 | 0 | 6 | 6 | 0 | 6 |
| | 1:250 | 5 | 1 | 4 | 6 | 6 | 0 |
| | 1:500 | 3 | 3 | 0 | 5 | 5 | 0 |
| o-Phenylphenol and p-tert. Amylphenol (p.c. 10) | 1:200 | 3 | 0 | 3 | 3 | 0 | 3 |
| | 1:500 | 8 | 1 | 7 | 2 | 0 | 2 |
| | 1:1000 | 6 | 6 | 0 | 2 | 2 | 0 |
| Iodophor (1.6% av. I) | 1:10 | 4 | 0 | 4 | 3 | 0 | 3 |
| | 1:50 | 4 | 2 | 2 | 3 | 2 | 1 |
| | 1:100 | 10 | 8 | 2 | 3 | 3 | 0 |
| | 1:200 | 9 | 9 | 0 | — | — | — |
| Benzalkonium Chloride (anhyd.) | 1:750 | 2 | 0 | 2 | — | — | — |
| | 1:1000 | 3 | 3 | 0 | — | — | — |
| Control | —— | 15 | 15 | 0 | 10 | 10 | 0 |

In another test it was found, however, that prior dilution of the influenza virus with 100 parts of saline raised the activity of the iodophore making it appear effective in a dilution of 1:213 (75 ppm av. I) in one case, and in 1:640 (25 ppm av. I) in another.

Anhydrous benzalkonium chloride inactivates influenza A virus in a concentration of 1:750, but not in one of 1:1000, under the conditions of this test.

The characteristic effect of organic matter merits the same consideration in the case of virucides, as it does in the case of germicides. What complicates the picture from the point of view of methodological relevance, is that influenza virus does not, of course, occur under practical conditions in the presence of allantoic fluid; the reduction of the antiviral activity by this fluid, and the extent of this reduction, merely serve to indicate the likelihood of a similar impairment by other

* The allantoic fluid used in these tests contained $10^7$ ID/50 per milliter. Equal parts, viz., 0.2 ml. of this fluid and of diluted disinfectant were mixed and held for 10 minutes at 20°, then 3.6 ml. of sterile distilled water was added, and 0.1 ml. of this mixture was injected into the allantoic cavity of a 10 day old embryonated egg; thus each egg received 50,000 ID/50. After incubation for 48 hours, the allantoic fluid was harvested and tested for the presence of virus by hemagglutination (60).

forms of organic matter to be encountered in connection with disinfectant practice.

While a number of virus infections are spread by arthropods, others are contracted via the gastro-intestinal or respiratory routes. Accordingly, the ideas advanced concerning the importance of a disinfectant's ability to maintain an enduring antibacterial potential on disinfected surfaces apply also in the case of those viruses which possess the demonstrated capacity of retaining their infectivity over a period of time. Relevant to this subject is the observation made by Parker, Dunham and MacNeal (53) according to which spray-dried influenza A virus retains its activity for 4, and more rarely, for 15 days; however, from an epidemiological point of view it is highly significant that the addition of 0.25 per cent of mucin permits this virus to survive for periods up to 6 weeks. This, of course, suggests the need of supplementary employment of dried influenza virus in the presence of mucin as test material for the evaluation of disinfectants, in view of the character of the mucus laden viral matter which occurs under the conditions of practice following its dispersal by influenza patients or carriers. Experiments carried out in our laboratory some time ago (unpublished) demonstrate the capacity of the synthetic phenolic disinfectants (referred to in Table VI) to inactivate dry influenza virus A and B, and thus to contribute to the control of the "secondary reservoirs" of respiratory virus infection of this origin.

It should be added at this point that influenza virus may be readily inactivated by different substances, as has been shown by a variety of testing methods (54-56). This is in contrast to the virus of poliomyelitis whose inactivation presents a more difficult problem. As indicated before, one must distinguish here between the effect upon relatively pure strains and that upon strains occurring in the presence of natural contaminants. It so happens that "clean" poliomyelitis virus is susceptible to the action of typical protein reactants, such as formaldehyde, hypochlorite, potassium permanganate, iodine and the like. With this as a premise, it must be assumed that because of the indiscriminate reactivity of these agents, the accompanying organic matter which, too, is mostly of protein character, will use up the reactive antiviral material with the same avidity as the virus itself; as a result only a fraction of the inactivating substance may remain available for the reaction with, and the inactivation of the polio virus.

That this is likely to be the case in practice may be concluded from the observed fact (57) that only a 1:50 dilution of a Type 1 polio virus suspension is inactivated by an iodophore concentration corresponding to 50 ppm of available iodine; neither the latter concentration, nor one corresponding to 75 ppm of iodine is capable of inactivating a 1:5 dilution of this virus suspension. While no experimental data are available in regard to still lower virus dilutions, there is hardly any doubt that the activity of a reactive substance of this type would decrease rapidly in response to the increase in the organic load. This is why claims for poliocidal action should be made as well as received, with caution; a demonstrated inactivation of a substantially pure polio virus suspension by a particular reactant need not necessarily constitute valid evidence in support of its fitness for use as a practical poliocidal disinfectant.

Different types of viruses are discharged by patients or carriers through nasopharyngeal secretions, also in urine and feces. This must lead, of necessity, to environmental contamination of surfaces from which infection is likely to spread by contact, by inhalation of dried, virus bearing fomites, etc. Although disinfection might play an important part in controlling or in reducing this infection hazard, only scanty information is available concerning the efficacy of the different available disinfectants as inactivators of the several pertinent viral pathogens.

These few remarks will suffice to show that the methodology of inquiry into the virucidal properties of disinfectants is in its initial stages, as regards both the mandatory delineation of their effectiveness against particular viruses, and the experimental conditions under which the tests should be performed in order to yield results paralleling or approaching those desired of practical virucidal disinfection.

For the sake of a general orientation, reference should be made to the chapter on "Virucidal Agents" by W. G. Dunham (58). A recent paper by Gershenfeld (59) contains valuable specific information on iodine as virucide.

## Summary

Under the heading of disinfectants, the paper considers the inadequacy of the "phenol coefficient" testing procedures, and some of the more recent attempts to overcome this obstacle by means of supplementary techniques.

The problem of sporicidal action of chemical agents, and particularly of formaldehyde germicides, is reviewed in the light of some recent data with special reference to the factor of unrecognized sporostasis affecting the claims for the sporicidal performance of such materials.

The direct relevance of "disinfection" to tuberculocidal action is emphasized, particularly with respect to hospital practice.

The ability of disinfectants to maintain an enduring antibacterial potential upon disinfected surfaces appears to have been neglected, yet it is deemed to be an important factor in their evaluation, from the viewpoint of controlling a significant portion of air-borne and environmental transmission of infection.

Under the heading of antiseptics, it is pointed out that there are no short cuts to their comparative assessment. So called standard methods at best represent preliminary screening procedures suitable for the separation of potentially useful preparations from totally useless ones. Reference is made to the neutralization or reversal of antiseptic action as a result of contact with biological materials, and the role of these factors in the evaluation of antiseptics. Screening tests of a series of liquid antiseptics by the "semi-micro" method involving the use of blood yields more representative results than are obtainable by the so-called F. D. A. testing procedure.

As to antifungal agents, there exist standardized procedures for the evaluation of their performance on inanimate surfaces. The same is not true of therapeutic antifungals, i.e., those for use on the skin. Brief reference is made to several factors involved in keeping this subject in the controversial area, particularly with respect to the transmission of fungus infection by contaminated surfaces.

Although the control of the propagation of several important virus diseases constitutes a logical objective of chemical disinfection, considerable fundamental and methodological information must be gathered before disinfection can achieve here the same rational position which it now holds with respect to controlling infectious disease of bacterial origin.

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# SULFONAMIDE HYPOGLYCEMIC AGENTS

EVER since the original report by Banting and Best with regard to the specific value of insulin in diabetes, a search has been made for some drug that would have an effect when given by mouth. In recent months, considerable interest has been aroused by the experimental trial of certain sulfonamides as hypoglycemic agents. Here, we shall try to explain their present status and future outlook.

## Nature and Role of Insulin

Insulin is a polypeptide hormone elaborated in the beta cells of the islets of Langerhans in the pancreas. At present, all insulin is extracted from the pancreas of animals, although its amino-acid composition and much of its precise molecular structure is now known. The mechanism whereby insulin exerts its hypoglycemic effect is not clearly established. Some biochemists believe that it acts by inhibiting certain hexokinase inhibitors leading to a better utilization of glucose. Hexokinase inhibitors, such as growth hormone and adrenal cortical hormones, are known to be present in the body and sometimes to cause hyperglycemia. Others believe that insulin acts by assisting in the transport mechanism of glucose through the cell membrane. In the event of a relative deficiency of insulin, glucose accumulates in the blood and the chain of events characterizing diabetes mellitus takes place: hyperglycemia, acidosis, ketonemia, coma, and, eventually, death. It is, of course, the disturbance in fat metabolism that leads to the acidosis, ketonemia, and coma rather than the accumulation of glucose itself.

Insulin cannot be given orally since, being a polypeptide, it is rapidly split by the proteolytic enzymes of the gastrointestinal tract. In this respect, it is like other polypeptide hormones such as corticotropin, pitocin, and others from the pituitary. The workers who preceded Banting and Best, while they believed that the pancreas contained insulin, could not extract it. The proteolytic enzyme trypsin, also found in the fresh pancreas, destroyed it before it was extracted.

There are many excellent insulin products today, some with a fairly rapid effect and others with a prolonged action. All, however,

must be injected. Many patients object to this procedure and it is, of course, true that no injection technique is as simple or acceptable as is the swallowing of a capsule or tablet.

## Oral Drugs

Over the years, many drugs have been found to produce a hypoglycemic effect when given orally. In almost every instance, the lowering of blood sugar was a direct toxic effect, usually on the liver. Obviously, such drugs could not be used in the control of diabetes.

In 1941, M. Janbon and his associates reported that the compound *p*-amino-sulfonamido-isopropyl-thiadiazole lowered blood sugar in man. This compound was known under the experimental code numbers VK57 and 2245RP. At the time, it was being investigated for its antibacterial effect. Later, other workers showed that a long series of *p*-amino-sulfonamido-alkyl thiadiazoles possessed a hypoglycemic effect and that the butyl, isobutyl, and amyl derivatives were even more active. Chen and Anderson in the United States studied the cyclopropyl derivative and reported the reversal of its hypoglycemic effect by alloxan.

## Other Sulfonamides

In 1954, two German workers studied the antibacterial action of a somewhat different but related sulfonamide, 1-butyl-3-sulfanilylurea (BZ-55). In trying this drug on themselves, they noted the classical signs of hypoglycemia: fatigue, hunger, perspiration, and trembling. This was shown by blood sugar determinations to be caused by the drug's hypoglycemic action. Clinical trials on diabetics were then carried out in Neumünster and Hamburg. Additional clinical experience has since been obtained with this substance under the name Carbutamide in the United States.

A closely related substance, 1-butyl-3*p*-tolylsulfonylurea, also known as Orinase, has been tried clinically under the auspices of the Upjohn Company. Both Orinase and Carbutamide appear to be quite similar in action.

## Pharmacology

These drugs are quickly absorbed when given by mouth, with maximum blood levels occurring within 3 to 6 hours. In 2 to 3 hours

following an oral dose, the blood sugar begins to drop. It is possible with sufficiently large doses to produce convulsions in test animals similar to those seen in insulin shock. These are relieved by the administration of glucose. The oral daily dose appears to be somewhere between 1 and 2 Gm. In mice, the LD50 dose orally is 11.5 Gm./Kg. so that there seems to be a good margin of safety, at least from the standpoint of acute toxicity.

While these drugs produce hypoglycemia in all normal humans, not all diabetics respond in this manner. It is now known that there must be some endogenous insulin present for these agents to have an effect. This is in agreement with the work of Chen who showed that if all the insulin producing beta cells of the pancreas are destroyed by the chemical alloxan then these sulfonamides do not lower blood sugar. Loubatières has also shown the drugs to be without effect in depancreatized dogs.

## Mechanism of Action

The manner in which these sulfonamides lower blood sugar is not yet established. One such drug has been reported as causing severe degenerative changes in the alpha cells of the pancreas. It is these same alpha cells which elaborate the hyperglycemic substance glucagon. Unfortunately, the role of glucagon itself in normal and diabetic persons is not clear, although glucagon does have a demonstrable hyperglycemic effect.

Loubatières concluded that these sulfonamides caused hypoglycemia by stimulating insulin secretion but it has been suggested by other workers that they act as insulinase inhibitors thus conserving and intensifying the endogenous insulin in the patient. This would explain the lack of action of these drugs in alloxan-treated dogs which produce no endogenous insulin at all. It would also explain the failure of these sulfonamides to produce an effect in certain long standing cases of severe diabetes unless some insulin is given concurrently by injection.

## Clinical Experience

German workers using BZ-55 (Carbutamide) found it of no value in young diabetics but effective in 25 of 28 older diabetics who previously were either untreated with insulin or inadequately treated. The blood sugar in most of these decreased rapidly to levels below

150 mg./100 ml. In some of these cases, the drug was discontinued after 10 days with no relapse ensuing for 3 months providing the diet was controlled. In a group of 38 patients previously on insulin, 28 were able to discontinue insulin on treatment with the sulfonamide. This same drug was tried in a clinic in the United States on a number of patients. The dose given was 2.5 Gm. the first day, 1.5 Gm. the second, and 1 Gm. daily thereafter.

In some cases, Carbutamide alone controlled the hyperglycemia; in others, it permitted a reduction in the insulin given and, in a third type of patient, it was entirely unsatisfactory. The unsatisfactory results were obtained with young persons having "unstable" diabetes and those patients who required emeregncy treatment. It seemed fairly effective in the majority of cases of mild or moderately severe diabetes.

It seems quite certain that the sulfonamide hypoglycemic agents are of value only in patients having some endogenous insulin. The long standing case of severe diabetes is not likely to be benefited. The obese diabetic may by their use avoid the necessity of either insulin injections or a rigid diet. It is likely, however, that such obese diabetics are better treated by an insistence on a rigid diet. This in itself will control many such patients, their blood sugar remaining normal when they achieve and maintain normal weight.

## Side Effects and Toxicity

To date, no serious toxic effects can be definitely ascribed to these sulfonamides. Drug rash and drug fever of a minor nature have been reported. One patient developed a leukopenia after daily administration of 1 Gm. for 3 months. No crystalluria has been observed in the clinical trials. The close chemical relationship of these drugs to the sulfonamides used as anti-infectives warrants some concern, however, over possible long term chronic toxicity. This is particularly true insofar as blood dyscrasias are concerned.

## Future Outlook

Whether these oral sulfonamides will achieve a useful place in medicine for the control of diabetes is at present uncertain. Some clarification of their mechanism of action is quite important. If they act only by destroying the alpha cells of the pancreatic islets then

there would seem to be some serious question concerning their proper use in medicine. If, on the other hand, they merely prolong and intensify endogenous insulin and cause no tissue damage, they would be promising. Their close chemical relationship to the antibacterial sulfonamides should make us suspect possible long term chronic toxicity. Only time, study, and more clinical experience with them will answer these many questions. In the meantime, these oral sulfonamide hypoglycemic agents are interesting, for they may provide the long sought insulin substitute in selected cases.

# SELECTED ABSTRACTS

**The Treatment of Congestive Heart Failure with Aminometradine.** Platts, M. M., and Hanley, T. *Brit. Med. J.* No. 4975:1078 (1956). Aminometradine (Mictine) was administered to a group of 20 patients with congestive heart failure. The initial dosage was 400 mg. of aminometradine orally each day. If satisfactory diuresis was not obtained, the dose was increased to 800 mg. a day. If this still produced a poor response, Mersalyl injections were given in place of the oral aminometradine.

The authors indicated that a satisfactory therapeutic response was obtained in 8 patients. They lost from 3.4 to 7.2 Kg. of body weight, showed a cumulative increase in urine flow ranging from 1.2 to 12.3 L., and became completely free from edema. Five patients were materially benefited by the drug but not all edema was eliminated. The remaining five patients had a poor or negligible therapeutic response. Increasing the dose from 400 mg. to 800 mg. a day did not appreciably increase the therapeutic effect. When compared with Mersalyl in two patients, the increase in salt excretion and in diuresis was greater with Mersalyl than with aminometradine.

The maximum effect from aminometradine does not develop until the 3rd or 4th day of administration. Since the drug is apparently not completely excreted each day, the authors suggested that it would probably be advisable not to administer the drug on one or two days each week. Little is known of the mode of action of the drug, but it seems probable that it inhibits reabsorption of sodium and chloride by the renal tubules. The increased urine flow is probably an osmotic diuresis due to the increased salt excretion.

Aminometradine produced a good response in about half of the patients in this study. Although its effect appeared not to be as good as Mersalyl, the advantage of being effective by oral administration would suggest its usefulness in the treatment of congestive heart failure where an immediate diuresis is not urgently needed. Toxic effects on the gastro-intestinal tract were observed in some patients. Three patients had to discontinue the drug because of nausea and vomiting.

**Comparison of Cortisone and Aspirin in the Treatment of Juvenile Rheumatoid Arthritis.** Ansell, B. M., Bywaters, E. G. L., and Isdale, I. C. *Brit. Med. J.* No. 4975:1075 (1956). Twenty-five patients, selected at random, were given either cortisone or aspirin for the treatment of juvenile rhematoid arthritis. Juvenile arthritis was defined as arthritis in two or more joints which began before the age of 16. Thirteen patients received cortisone and 12 received aspirin.

Therapy was given in courses of 12 weeks with one week between without treatment. The usual cortisone dosage was 300 mg. the first day, 200 mg. the second, and then 100 mg. for 5 days. Following this, therapy was adjusted to the needs of each patient, usually between 25 and 200 mg. a day. The dosage for aspirin for older children was 6 Gm. a day for the first week, 2 Gm. a day for the second week, and then adjusted individually between 3 and 6 Gm. a day. Smaller children received proportionately less. Later, continuous therapy replaced the course therapy.

The results from therapy in the two groups showed little difference at the end of one year. Both groups showed approximately the same degree of clinical and functional improvement. The hemoglobin was slightly higher in the cortisone-treated group. Complications were few in both groups. Those complications encountered were mostly concurrent infections which had nothing to do with the drug therapy.

The results from this limited trial would not suggest that cortisone is superior to aspirin in the treatment of the rheumatoid arthritis of childhood.

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**Serum Concentrations and Urinary Excretion Following the Oral Administration of Novobiocin.** Wright, W. W., Putnam, L. E., and Welch, H. *Antibiot. Med. 2*:311 (1956). The serum concentrations and the urinary excretion following the oral administration of novobiocin was studied in 60 normal, healthy, adult men. The antibiotic was administered in single doses of 0.25, 0.5, 1.0 and 2.0 Gm. or in multiple doses of 0.5 or 1.0 Gm. at 12 hour intervals until six doses had been given.

Following the administration of single doses of the antibiotic very high blood cencentrations were obtained. Following a single dose of

0.25 Gm. the average peak serum level was 10.9 μg./ml., following 0.5 Gm. it was 18.8 μg./ml., following 1.0 Gm. it was 42.4 μg./ml., and following 2.0 Gm. it was 67.7 μg./ml. With multiple doses, after the fourth dose of 0.5 or 1.0 Gm. the serum concentration was about the same as that obtained from a single dose of 1.0 or 2.0 Gm., respectively. After the fifth dose a somewhat higher peak was obtained but after the sixth dose the peak serum concentration was about the same as that following the fourth dose. Following the last multiple dose the novobiocin disappeared from the blood at about the same rate as after the single dose.

The authors stated that on the basis of the sensitivity of susceptible organisms and the blood levels obtained with this antibiotic, it would appear that doses of 0.5 Gm. twice a day should provide adequate therapeutic blood levels.

The urinary excretion of novobiocin was quite low. There was some evidence that as the blood concentration increased the urinary concentration also increased. However, the total excretion over the dosages administered ranged from 2.7 to 3.3 per cent of the dose administered.

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**A Study of the Effects of Various Factors on the Consistency of Paste Cream Shampoos.** Patterson, R. L. *Drug and Cosm. Ind.* *78*:322 (1956). The variation in the consistency of paste cream shampoos has been troublesome. Several factors were found to affect the consistency of a cream having the general formula:

| | |
|---|---|
| Sodium alkyl sulfate paste | 70-80 per cent |
| Sodium stearate | 6-9 per cent |
| Stearic acid | 0.5-2.0 per cent |
| Sodium sulfate | 0-3 per cent |
| Lanolin | 0-2 per cent |
| Perfume, preservative, etc. | As desired |
| Water | Remainder |

The glossy, soft product was found to be due to the formation of many small crystals which were too short and insufficiently aggregated. The curdy product was the result of the formation of large crystal aggregates with free liquor between them. Properly formed crystals

were fine to moderately course, forming a mat which did not break under its own weight. The crystals can exist in two distinct phases. Phase I existed between about 85° F. and the melting point of the crystals, i.e., 120° to 130° F. Phase II developed between about 80° and 95° F. and varied all the way from fine plates to long fibers. The rate at which the transition from phase I to phase II occurred controlled the crystal growth and determined the subsequent consistency behavior.

The paste shampoo was ordinarily prepared at a temperature of about 170° F. Quick cooling, about 15 minutes, to 100° F. greatly improved the consistency of the product as compared with longer cooling. Agitation during cooling speeded up the cooling rate, maintained a more uniform temperature throughout the mass, improved smoothness, and caused break-up of larger crystal masses. The storage temperature during the tempering period, the first 24 hours after packing, exerted a pronounced effect upon the end product. Tempering at 80° F. produced the greatest uniformity of consistency. Tempering at 60-70° F. produced the glossy type of soft product. At 90° F., greater variations in consistency were obtained. During the tempering period, crystal growth was apparently substantially completed.

Formulation variation also produced changes in the consistency of the product. Sodium stearate increased the firmness, free fatty acid increased the softness, added electrolyte (sodium sulfate) increased the firmness up to about 3 per cent but above this caused softening, lanolin improved the smoothness and texture and produced some softening, and perfume may have an appreciable softening effect.

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**The Chemical Investigation of Poison Ivy.** Dawson, C. R. *Trans. of the N. Y. Acad. Sci. 18*:427 (1956). The chemical constituents of the irritating oleoresin obtained from *Rhus* varieties, particularly *Rhus toxicodendron radicans* (poison ivy), have been investigated for several years. The compounds were found to be largely phenolic in nature. One compound was found to have an olefinic side chain at the 3 position of catechol. When these substances were separated from the natural oleoresin they were found to be methylated compounds which were inactive with regard to vesicant properties. No method has been devised to demethylate the compounds without

destroying the double bond in the side chain. However, studies of the chemical nature of these compounds were more safely carried out when they were methylated, since the methylated form was essentially nonallergenic.

In recent years a crystalline saturated compound, 3-pentadecylcatechol (PDC), has been found to be a valuable standard agent for patch testing for sensitivity to poison ivy. PDC is also present in poison ivy oleoresin in very small quantities. Quite recently, it has been shown that PDC can be used successfully as a desensitizing agent. In more than 2,000 patients, intramuscular injections of PDC, given over a period of several weeks, caused persons sensitive to poison ivy to become refractory to the plant. This lower level of sensitivity could be maintained by 2 to 4 injections a year.

Further investigations are in progress on other components of the poison ivy oleoresin and upon the mechanism by which the dermatitis occurs. One present concept is that the alkyl and alkenyl catechols in the poison ivy principle form a complex with specific proteins of the skin when contact is made. This complex then initiates the events leading to the dermatitis typical of poison ivy.

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**The Topical Application of Hydrocortisone Powder in the Treatment of Hay Fever.** Herxheimer, H., and McAllen, M. *The Lancet* No. 6922:537 (1956). A group of 24 patients with severe hay fever who had failed to respond to other types of therapy were treated by the insufflation of 15 mg. of hydrocortisone powder each day. The hydrocortisone was mixed with 85 mg. of lactose and supplied in the form of a capsule for use in an insufflator. The powder was applied as a spray three times a day, the entire 15 mg. of hydrocortisone being used each day. In many cases the blockage of the nasal passageway was so complete that 0.02 per cent naphazoline had to be applied initially to open the air-way. This pre-treatment was discontinued as soon as the air-way remained open. The hydrocortisone was applied for five days and then stopped. If relapse of symptoms occurred, the hydrocortisone was again used.

The authors reported that complete control of the symptoms was obtained in 23 of the 24 patients within 10 days, and most of them responded dramatically within 48 hours. The slower responses occurred in those patients with profuse rhinorrhea, which prevented more than small amounts of the drug from remaining on the mem-

branes except for brief periods. Nineteen of the patients were followed for the entire season. Only 2 of these patients relapsed while taking hydrocortisone. Nine patients maintained control of their symptoms by taking hydrocortisone throughout the season. The remaining 8 patients discontinued treatment after from 5 to 20 days without a return of more than slight symptoms. In 20 of the 24 patients, eye symptoms associated with rhinitis were controlled. Also, in 4 of 10 patients who normally had pollen asthma, no asthma developed.

The authors pointed out that the safety of this method of treatment provides a significant advantage over desensitization therapy. They stated that hydrocortisone in a dosage of 15 mg. a day applied topically to the nasal membranes could not have any harmful effects even if completely absorbed during the 8-week hay fever season.

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**Preliminary Evaluation of Pulp Reaction to Ultrasonic Cavity Formation.** Healey, H. J., Patterson, S. S., and Van Huysen, G. *U. S. A. F. Med. J. 7*:685 (1956). The ideal instrument for cavity preparation should be capable of being easily manipulated and precise, noiseless, painless to the patient, nonthermogenic to the tooth, and nontraumatic to the pulp and periapical tissue. At present, no one instrument will fulfill all of these requirements, according to the authors. The recent availability of an instrument utilizing high-frequency vibrations for the preparation of tooth cavities raised the question of the effect of these vibrations upon the tooth pulp. The authors investigated this effect.

Noncarious maxillary and mandibular premolar teeth which were scheduled for removal for orthodontic purposes had cavities prepared in them by using either a steel bur, a diamond stone, or the ultrasonic instrument. The cavities thus formed were restored with a calcium hydroxide base covered with zinc oxyphosphate cement or amalgam.

During the postoperative period there were no manifestations of unfavorable symptoms such as constant pain or temporary hypersensitivity in any of the teeth. One to two months after being prepared and restored, the teeth were extracted using local anesthesia. The teeth were preserved in formaldehyde and then examined histologically. No unfavorable pulpal reaction was found resulting from the use of an ultrasonic cutting instrument. The reactions of the pulp to the three cutting instruments were very similar.

**The Value of the Antenatal Administration of Iron.** Edgar, W., and Rice, H. M. *The Lancet* No. 6923:599 (1956). The disproportionate increase of plasma volume during pregnancy often causes a gradual fall in the hemoglobin level as pregnancy advances. This hydremia of pregnancy is often associated with a minimal intake of iron. The authors investigated the effect of the administration of 9 gr. of ferrous sulfate a day on this hydraemia on a group of 89 patients.

Ferrous sulfate was chosen because ferrous gluconate was not available at the time of the study. Enteric coated tablets were not used because it was felt that the greatest absorption of iron occurs from the stomach and the upper intestine. White sugar-coated tablets were used to overcome the psychological effect of the usual green tablets.

The hematological tests employed were the hemoglobin level, the red cell count, the color index, the packed-cell volume, the mean corpuscular hemoglobin concentration, and the mean corpuscular volume. Blood specimens were taken every 4 weeks.

It was found that only 8 women were excluded from the test because of intolerance to iron. Of the 89 patients receiving iron, 69 maintained satisfactory hemoglobin and other hematological indices thoughout pregnancy. The remaining 20 patients failed to respond to the low-level iron administration employed. The authors stated that the routine determination of the hemoglobin level was a useful guide to the hematological condition of the patient. Where the hemoglobin was found to be low, other values were also low. They recommended that a routine hemoglobin determination be made at the initial visit and again at the 32nd to 36th week of gestation. Further hematological investigation should be required in only a few cases where complications are suspected.

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